AGROCHEMICAL METABOLITES

Synthesis, purification and characterisation of agrochemical metabolites

www.hyphadiscovery.com

HYPHA'S ONE-STOP METABOLITE SHOP

Hypha's One-Stop Metabolite Shop enables access to mammalian, plant and soil derived metabolites to support definitive regulatory studies, as part of the registration requirements for agrochemicals.

We use chemical synthesis, microbial biotransformation, mammalian tissue fractions (multiple species of S9s and microsomes) plus recombinant enzymes such as PolyCYPs and glycosyltransferases.

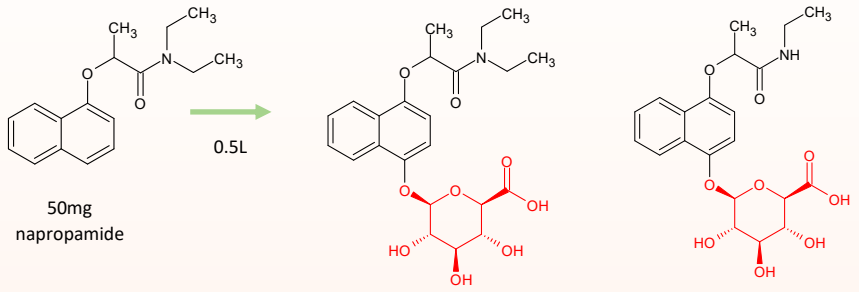
- **Oxidised and reduced metabolites**
- **Conjugated metabolites, including *O*-, acyl, *N*- & *N*-carbamoyl glucuronides, glucosides & sulfates.**
- **Multiple metabolites and multi-step metabolites**
- **Purification of metabolites and manufacturing impurities**
- **Structure elucidation by cryoprobe NMR spectroscopy**
- **Provision of Certificates of Analysis including qNMR**
- **Scalable to multi-gram amounts**
- **Formulation know-how for poorly-soluble compounds**
- **Cold, stable-labelled and radiolabelled metabolites**

For more information or to discuss a project email us at:

enquiries@hyphadiscovery.com

Microbial biotransformation

Many of Hypha's microbes are derived from soil. They undertake oxidised and reduced metabolic reactions, as well as being effective for making conjugated metabolites. Reactions are fully scalable. Using this approach, it is also possible to obtain metabolites formed from multiple sequential reactions in a single incubation, e.g. hydroxylation and subsequent glucuronidation.



Rat metabolites of the herbicide napropamide formed in "one pot" by microbial biotransformation via hydroxylation and subsequent glucuronidation and *N*-dealkylation.

Late-stage chemical synthesis

Our late-stage chemical methods are effective for synthesis of all types of glucuronides, glucosides and sulfated metabolites. Reactions are fully scalable to supply gram amounts, and are a proven and cost-effective way to access conjugated metabolites. Oxidised metabolites may also be accessed through application of a broad range of chemical oxidation conditions.

Chemical and biotransformation methods can be used in parallel to provide multiple metabolites, such as in one client project where four glucose conjugates of an agrochemical were required. Two different chemical synthesis methods were used to make three of the conjugates, with the fourth accessed using one of Hypha's recombinant glycosyltransferases, at over 70% conversion.

Mammalian biotransformation

We use multiple species of S9s and microsomes from liver and other tissues to make human and other mammalian metabolites that are more difficult-to-synthesise using other routes.

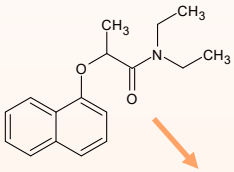
Purification and structure elucidation

In addition to synthesising metabolites, we can also purify them direct from biological matrices such as plasma, urine and faeces.

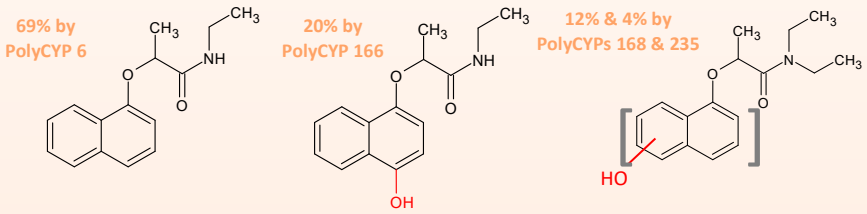
Hypha provides rapid and unambiguous structural identification through access to a 700MHz NMR spectrometer equipped with a 1.7mm micro-cryoprobe. This means only small amounts of metabolites are needed to acquire data sets for full structural elucidation. Our scientists are experts in data interpretation.

Recombinant enzymes

We have a number of recombinant enzymes for making phase 1 metabolites. Our PolyCYPs® enzymes have been mined from soil derived bacteria, providing a diverse set of microbial CYPs effective for producing CYP-derived metabolites.

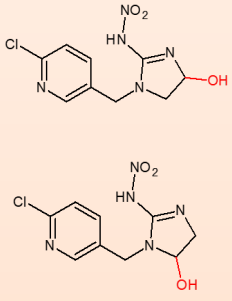


PolyCYPs+ kits contain 20 enzymes. In addition to 18 PolyCYPs isoforms, the kit contains human aldehyde oxidase (AOX1) and the main human hepatic flavin-containing monooxygenase (FMO3), with the other human FMO isoforms also available at Hypha.



Metabolites produced by PolyCYPs involving desethylation and aromatic ring hydroxylations

Reactions are scalable either by resupply of lyophilised enzymes for mg scale production in-house, or larger scale production up to gram scale at Hypha, with optional purification and structure elucidation.



PolyCYPs and microbes can be used in a complementary way to access multiple metabolites, such as the main hydroxylated mammalian metabolites of imidacloprid. Following screening of imidacloprid against a panel of microbes and PolyCYP enzymes, a fungal species was used to produce 4-hydroxyimidacloprid. Conversely a bacteria derived enzyme, PolyCYP 486, was more efficient at making 5-hydroxyimidacloprid. The positional isomers were separated, confirmed by NMR spectroscopy and supplied for use as analytical standards.

PolyCYPs kits can also be used for creating radiolabelled metabolites for direct comparison with the radio profiles from mass balance and distribution study samples. PolyCYPs provides a route to scalable access to the CYP derived metabolites in these samples for definitive MetID and tox studies.

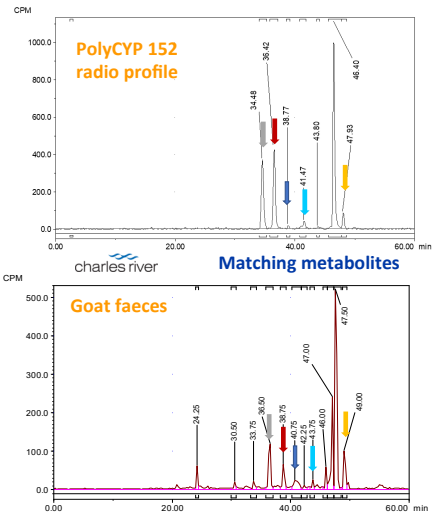
Client feedback

Director, US Client

"Hypha Discovery has been a valuable metabolite ID partner*. They *have provided answers to some of our most challenging metabolism and metabolite ID problems. We really appreciate the breadth of expertise available at Hypha Discovery and will definitely reach out for future work."

Principal Research Chemist, Syngenta

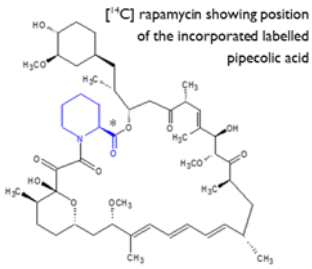
"We have used Hypha Discovery to provide samples of several pesticidal natural products by fermentation. We find them good people to work with. They provide a flexible and reliable service, meeting agreed deadlines, and so far, have always provided us with the target compounds. We are pleased to recommend Hypha Discovery."



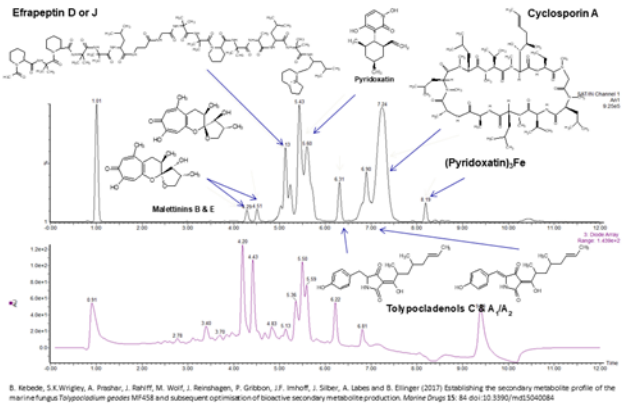
Radiometric analysis and comparison of PolyCYPs incubations of a 14C-labelled agrochemical with a goat faeces sample from a mass balance study. High resolution LC-MS/MS showed oxidation of cycloalkyl and aromatic carbons of this compound best by PolyCYP 152.

Other Services—Natural Products

Generation, purification and NMR characterisation of known and novel microbial products



Metabolite profile of *Tolypocladium geodes*



B. Kebede, S.K.Wrigley, A. Prosser, J. Rahiff, M. Wolf, J. Reinshagen, P. Gribbon, J.F. Imhoff, J. Silber, A. Labes and B. Ellinger (2017) Establishing the secondary metabolite profile of the marine fungus *Tolypocladium geodes* MF458 and subsequent optimisation of bioactive secondary metabolite production. *Marine Drugs* 15: 84 doi:10.3390/md15040084

Contact us

For more information or to discuss a project email us at:

enquiries@hyphadiscovery.com

Hypha Discovery Limited
154B Brook Drive, Milton Park
Abingdon, OX14 4SD, UK

www.hyphadiscovery.com